Surface Passivation for Molecular Motor Protein Assays

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"Kiney"

Open Notebook Science

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We would also like to thank Susan Atlas, PI of the DTRA project.

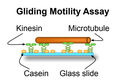
Motivation

Kinesin and microtubules have been proposed as components for chemical and biological sensors. In order to fully understand the dynamics of kinesin and microtubules, fundamental questions must be answered related to how we observe those interactions. One way to observe their interactions is by using a ***gliding motility assay*** which can easily be visualized as a molecular "crowd surfing" for microtubules. In order for kinesin to support microtubule crowd surfing, the glass the kinesin is on must be functionalized in order to passivate the surface. Understanding the interactions of kinesin with this passivation is crucial to understanding novel ways to engineer MEMS devices for the detection of chemical and biological materials.

Introduction

Surface passivation is crucial for kinesin and microtubule experiments. Without passivation, gliding motility assays will fail. The current beliefs as to why failure occur include[1]:

- No structural support for kinesin.
- Kinesin denatures when it hits glass.
- Motor domains may inactivate when they contact glass.



To run experiments, a protein called ***casein*** is used to passivate the glass. There are many issues with using casein, some of them include:

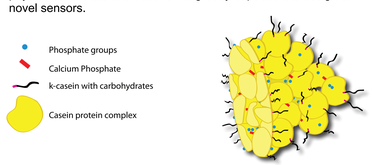
- It is designed to carry calcium phosphate to infant mammals. This calcium could aid in depolymerizing microtubules
- No crystal structure exists.
- Its use is based on legacy.

Casein[2]

- Globular protein.
- Makes up 80% of the protein content in bovine milk.
- Consists of 3 major subunits, α, β, κ.
- Carries calcium phosphate to infant mammals.

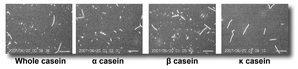
* Amphiphilic.

An issue that makes casein unattractive as a surface passivator is that it is designed to deliver calcium to infant mammals. Calcium is a known depolymerizing agent for microtubules. Its use is also based on legacy which has not been fully investigated. To fully elucidate the physics behind kinesin and microtubule interactions a simpler, cleaner surface passivator must be found. Knowing the physics behind the interactions will greatly expedite the design of novel sensors.

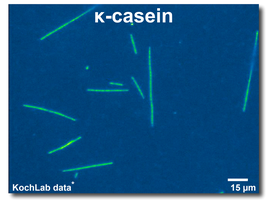


Surface passivation with casein

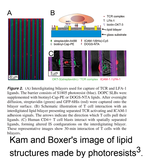
- Glass must be passivated to support a gliding motility assay.
- Different components of casein support microtubules differently.
- There is no consensus in the scientific community as to why casein supports gliding motility assays.
- The differences between caseins suggest we can find something else that supports a gliding motility assay.



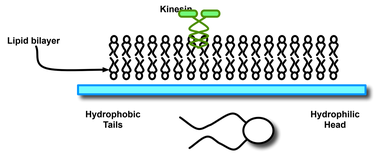
Very nice work done by Verma et. al. Scale bar is 10 μm[1].



Here we confirmed that κ-casein does support longer microtubules (green lines) in a gliding motility assay as Verma et. al. stated[1].



Kam and Boxer's image of lipid structures made by photoresists[3].



Surface passivation with lipids

- Lipids could be a cleaner method of surface passivation.
- Crystallizable, functionalizable, and can be synthetic.
- Spin coating lipids can make single to multiple lipid layers.
- Liposome bilayer formation.

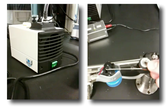
There are many different types of lipids available. They range from size, to charge, to natural or synthetic. You can also functionalize the head and tail groups with just about anything you want. The best part about using lipids is that you can pattern them on photoresist to make any structure you want.

Lipid slide preparation

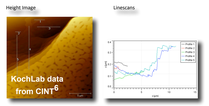
- Spin coating.
- Bilayer formation.

Depending on what chemistry we are interested in looking at, we can either spin coat lipids[4] on either a hydrophobic or hydrophilic glass slide and make lamellar bilayers of lipids. By varying the amount of lipids spin coated on a slide, we can generate a single layer of lipid molecules that support aqueous environments.

Spin Master Micro
DIY evaporator made from a DC motor controller, switch, and a model train motor. For more information on DIY bio, and links to other DIY projects, please see the Wikipedia article on DIYbio.



Spin Coater 3000
Transforming the Spin Master Micro into a spin coater.



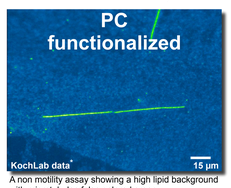
AFM of spin coated lipids
Indicates we can make lamellar structures from spin coating.

Glass surfaces
Silanized glass.

Liposome
Used for making bilayer substrates.

Preliminary results

Use of phosphatidylcholine (PC) on both hydrophobic and hydrophilic slides have given negative results. While a lipid layer can be formed on the substrate, no motility was observed.



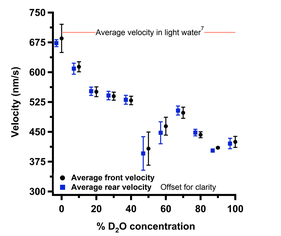
A non motility assay showing a high lipid background with microtubules false colored green.

Future work with lipids and heavy water

- ***Phosphatidylethanolomine.*** PE has a higher melting temperature than PC and thus, may give kinesin a solid support for motility.
- ***Charged lipids.*** There exists a possibility that kinesin is attracted to a charged group on the casein. Determining what charge, positive or negative, is easily done by functionalized lipids.
- ***Cysteine functionalized lipid head groups.*** Casein has many cysteine residues on it. There is a possibility that the reason kinesin sustains motility with casein is because it sticks to the cysteine residues and prevents the motor domains of kinesin from sticking to the glass.

In parallel with this lipids work, we have been studying the affects of heavy water and osmotic stress on kinesin motility. Preliminary data to the right shows a distinct effect of D_2O on gliding speed, errors are SEM. We have also seen a long-term stabilization of the gliding assay. It is possible that heavy water will improve passivation and stabilization of kinesin, and we will explore its effects in combination with studies of surface functionalization by caseins, lipids, and other molecules.

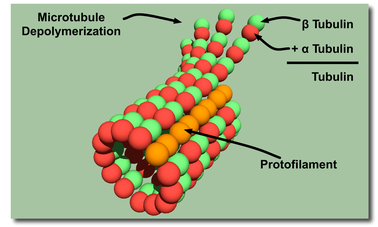
For more information about the experiments, please see Maloney's open notebook at: **http://www.openwetware.org/wiki/User:Andy_Maloney**



Please see the poster on Wednesday (W236) for more information.

Microtubules

- Heterodimer of tubulin subunits α and β. One α and one β subunit together is called tubulin
- Tubulin forms polymers called protofilaments.
- Microtubules are made from 13 - 17 protofilaments.
- They are hollow and are an average of 25 nm in width.
- Calcium causes depolymerization.



Kinesin

- Dimer that consists of two heavy chains and two light chains.
- The heavy chains form the "head" group or the motor domains.
- The light chains form the "tail" group where cargo binds. The kinesin supplied to us from Dr. Liu does not have light chains. It is a truncated heavy chain Drosophila kinesin-1.
- The chains are connected by a "neck linker" and an intertwined stalk region.
- Uses ATP to generate motion.
- Please see the poster on Wednesday (W236) for more information on kinesin's kinetic cycle.

HHMI report[5].

Acknowledgments

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- Gabriel Montano (CINT)
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References

1. Verma, Vivek, William Hancock, and Jeffrey Catchmark. "The role of casein in supporting the operation of surface bound kinesin." Journal of Biological Engineering 2, no. 1 (2008): 14.
2. http://www.food-info.net/uk/protein/milk.htm
3. Kam, Lance, and Steven G. Boxer. "Spatially Selective Manipulation of Supported Lipid Bilayers by Laminar Flow: Steps Toward Biomembrane Microfluidics†." Langmuir 19, no. 5 (March 1, 2003): 1624-1631. doi:doi: 10.1021/la0263413.
4. Mennicke, Ulrike, and Tim Salditt. "Preparation of Solid-Supported Lipid Bilayers by Spin-Coating." Langmuir 18, no. 21 (October 1, 2002): 8172-8177. doi:doi: 10.1021/la025863f.
5. HHMI Report 2005.
6. CINT is a user facility. More information can be found here, http://cint.lanl.gov/
7. Guydosh, Nicholas R, and Steven M Block. "Direct observation of the binding state of the kinesin head to the microtubule." Nature 461, no. 7260 (September 3, 2009): 125-128. doi:10.1038/nature08259. Supplemental information.

* Images false colored in ImageJ.